

CLAIMS

What is claimed is:

1. An isolated nucleic acid fragment encoding a TmoST polypeptide selected from the group consisting of:
  - 5 (a) an isolated nucleic acid fragment encoding the amino acid sequence selected from the group consisting of SEQ ID NOs:113, 114, and 115;
  - (b) an isolated nucleic acid fragment encoding two polypeptides TmoS of at least 973 amino acids having at least 83% identity, and TmoT of at least 220, having at least 85% identity based on the Smith-Waterman method of alignment with the amino acid sequence selected from the group consisting of SEQ ID NO:113, 114 and 115;
  - 10 (c) an isolated nucleic acid that hybridizes with (a) or (b) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; and
  - (d) an isolated nucleic acid fragment that is complementary to (a), (b), or (c).
- 20 2. The isolated nucleic acid fragment of Claim 1 selected from the group consisting of SEQ ID NOs:113, 114, 115.
3. A polypeptide encoded by the isolated nucleic acid fragment of Claim 1.
- 25 4. The polypeptide of Claim 3 selected from the group consisting of SEQ ID NO's:116 and 117.
5. A chimeric gene comprising the isolated nucleic acid fragment of Claim 1 operably linked to at least one suitable regulatory sequence.
6. A host cell transformed with the chimeric gene of Claim 5.
7. The host cell of Claim 6 wherein the host cell is a bacterium.
- 30 8. The transformed host cell of Claim 7 wherein the host cell is selected from the group consisting of *Pseudomonas*, *Burkholderia*, *Acinetobacter*, and *Agrobacterium*.
9. A method of obtaining a nucleic acid fragment encoding a TmoST polypeptide comprising:
  - 35 (a) probing a genomic library with the nucleic acid fragment of Claim 1;
  - (b) selecting for a DNA clone that hybridizes with the nucleic acid fragment of Claim 1; and

- PCT APP - INVENTION
- (c) sequencing the genomic fragment that comprises the clone identified in step (b), wherein the sequenced genomic fragment encodes a TmoST polypeptide.
10. A method of obtaining a nucleic acid fragment encoding a bacterial TmoST polypeptide, the method comprising:
- 5 (a) synthesizing at least one oligonucleotide primer corresponding to at least a portion of a sequence selected from the group consisting of SEQ ID NOs:113, 114, and 115; and
- 10 (b) amplifying an insert present in a cloning vector using the oligonucleotide primer of step (a),wherein the amplified insert encodes a TmoST polypeptide.
11. The product of the method of Claims 9 or 10.
12. A method for the production of *p*-hydroxybenzoate, the method comprising:
- 15 (a) contacting a transformed host cell with a medium comprising,
- 20 (i) an aromatic organic substrate selected from the group consisting of; toluene, *p*-cresol, *p*-hydroxybenzyl alcohol, *p*-hydroxybenzaldehyde, and aromatic compounds degraded by the toluene monooxygenase enzyme pathway,
- (ii) at least one fermentable carbon substrate, and
- (iii) a nitrogen source;
- 25 wherein the transformed host cell is (1) lacking a *p*-hydroxybenzoate hydroxylase activity, and (2) comprises genes encoding toluene-4-monooxygenase, TmoX, PcuR, *p*-cresol methylhydroxylase, TmoST polypeptides and *p*-hydroxybenzoate dehydrogenase activities, each gene being operably linked to suitable regulatory sequences;
- 30 (b) incubating the transformed host cell for a time sufficient to produce *p*-hydroxybenzoate; and
- (c) optionally recovering the *p*-hydroxybenzoate produced in (ii).
- 35 13. The method of Claim 12 wherein the fermentable carbon substrate is selected from the group consisting of monosaccharides, oligosaccharides, polysaccharides, carbon dioxide, methanol, formaldehyde, formate, and carbon-containing amines.

14. The method of Claim 12 wherein the fermentable carbon substrate is glucose.

15. The method of Claim 12 wherein the transformed host cell is selected from the group consisting of *Pseudomonas*, *Burkholderia*,

5 *Acinetobacter*, and *Agrobacterium*.

16. The method of Claim 12 wherein the aromatic organic substrate is present in the medium in a concentration of less than 500 ppm.

17. The method of Claim 12 wherein the aromatic organic substrate is present in the medium from 30 ppm to 60 ppm.

10 18. An expression plasmid pMAX47-2.

19. The method of Claim 12 wherein the transformed host cell comprises plasmid pMC4 as shown in Figure 4.

20. The method of Claim 12 wherein the transformed host cell further comprises the genes encoding TmoST activity.